Histologic Grading and Prognostic Biomarkers in Salivary Gland Carcinomas

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Abstract: Both the variety and rarity of salivary gland carcinomas pose challenge for using histologic grade and biomarkers to predict outcome. Mucoepidermoid carcinoma is the histologic subtype for which grading is most prognostically and therapeutically relevant. This tumor is graded using standard schemes in a 3-tier manner with the intermediate-grade category shows the most variability between grading systems and thus the most controversy in management. The t(11;19)(q21; p13) *MECT1-MAML2* translocation may be an objective marker that can help to further stratify difficult cases. Adenoid cystic carcinomas are graded based on pattern with solid areas correlating with a worse prognosis. Occasionally, adenoid cystic carcinomas may undergo transformation to highly aggressive pleomorphic high-grade carcinomas with frequent nodal metastases. Comparative genomic hybridization has revealed several chromosomal regions (such as 1p32-p36, 6q23-q27) of prognostic interest in adenoid cystic carcinoma. Carcinoma ex-pleomorphic adenoma is actually a category of tumors rather than a single tumor type with both aggressive and indolent versions. These tumors should be further qualified as to type/grade of carcinoma and extent, as intracapsular and minimally invasive tumors behave favorably. Acinic cell carcinomas, although generally considered low grade, can recur, metastasize, or even prove lethal in a significant number of cases suggesting amenability to a grading scheme to separate these biologic groups. Although aggressive histologic parameters (anaplasia, necrosis, and mitoses) are predictive of poor outcome, a standard grading scheme does not yet exists. Acinic cell carcinomas can also undergo high-grade transformation.

Key Words: grade, salivary gland carcinoma, biomarker prognosis

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The predicament of approaching and managing a salivary gland carcinoma stems from the extreme diversity of tumor types, their relative rarity, and requirement for long-term follow-up in many instances to predict outcome.[1] For example, salivary gland carcinomas constitute only 3% to 5% of all head and neck malignancies. However, at least 24 different types are recognized by the World Health Organization (WHO).[2] It is understandable, then, that this would translate in to significant diagnostic challenges. In addition, however, these features also pose significant challenges to prognosis and treatment as it is critical to translate the various salivary gland carcinoma types into therapeutically meaningful categories as indicated in Table 1.[3,4]

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General clinical parameters such as stage (size, soft tissue or skin involvement, and nodal status), age, and margin status remain important prognosticators for salivary gland carcinomas.[5–7] However, histologic grade, and thus indirectly, tumor type, also rank highly as important prognosticators.[6,7] High-grade salivary carcinomas have a 5-year survival of roughly 40% whereas low and intermediate-grade tumors have a 5-year survival of 85% to 90%.[1,6] In large series, histologic grade is an independent predictor of outcome in multivariate analysis, but it also tends to correlate with other adverse prognosticators such as size and nodal status.[7] Other histologic parameters such as perineural and angiolymphatic invasion may also be useful prognosticators and are in some cases incorporated into grading schemes.[5–7]

However, many "outliers" exist within this framework of stratification suggesting that our standard approach to prognosis of salivary gland carcinomas can be further refined. Flaws in traditional grading systems stem from the lack of sufficient sample size to devise statistically valid systems for a specific tumor type. As such, grading of most salivary tumors is carried out "intuitively" using general cytomorphologic features (pleomorphism, mitoses, and necrosis) and is thus prone to lack of reproducibility or standardization. Furthermore, most tumor types are actually not routinely graded. This applies to tumors perceived to be definitionally "high risk" for aggressive behavior (ie, conventional salivary duct carcinoma, squamous cell carcinoma, and small cell neuroendocrine carcinoma) or "low risk" (ie, epithelial-myoepithelial carcinoma and polymorphous low-grade adenocarcinoma). The drawback to this assumption is that histologically and biologically aggressive variants of intrinsically low-risk tumors do exist as do more indolent versions of high-risk tumors. Despite the aforementioned limitations, this approach is still very useful in delineating aggressive from indolent malignancies.

In fact, for more common malignancies, grading systems have been developed and are fairly effective in prognosis. The 2 major named tumor types that are consistently graded in current practice are adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC). In addition, adenocarcinoma, not otherwise specified, and cystadenocarcinoma are also typically assigned a grade, although these tumors are not common enough to generate a formal system. Finally, grading of acinic cell carcinomas (AciCCs) is somewhat controversial. Typically considered a low-risk tumor, many studies suggest an unusually high rate of lymph node metastasis as compared with other low-risk tumors. In addition, histologic studies actually point to the ability to stratify these tumors based on cytomorphologic grading parameters[8,9] suggesting that a grading system is necessary for these tumors.

Table 2 divides all entities listed in WHO classification scheme into low-risk and high-risk categories based on a combination of entity defined behavior and grade.[10]

TABLE 1. General Categories of Management of Primary Salivary Gland Carcinomas[3,4]

Surgery Alone	Surgery and Radiotherapy	Additional Neck Dissection	Systemic Chemotherapy
Negative margins	Close (< 2 mm) or positive margins	All cN+	Metastatic or unresectable disease
Low-grade histology	High-grade histology	cN0 but high-grade histology	
Low risk (nonangioinvasive and noninfiltrative) histologic subtype	High risk (highly infiltrative) histologic subtype	cN0 but high risk (angioinvasive) histologic subtype	
Low T stage (T1 or T2)	High T stage (T3 or T4)	cN0 but high T stage (T3 or T4)	
	pN+		
	Perineural invasion*		

*Somewhat controversial depending on tumor type.
cN+ indicates clinically node positive; cN0, clinically node negative; pN+, pathologically node positive; T, tumor stage in TNM classification.

Ancillary tests such as biomarker immunostains, and more recently, molecular assays for key gene alterations have the potential to supplement the traditional parameters delineated above. However, to date, only a limited subset of markers show promise in augmenting the prognostic value of standard clinicopathologic parameters. Ultimately, a successful approach for improving the prognostication of salivary carcinomas should be based on the understanding that many of these tumor types have a unique biology that requires individual consideration; not all carcinoma types are created equal.

SPECIFIC ENTITIES

Mucoepidermoid Carcinoma

MEC is the most common salivary gland malignancy in adults and children and is histologically comprised of a mixture of mucus cells, intermediate cells, and squamoid or epidermoid cells. MEC was formalized as a distinct entity by Stewart et al[11] in 1945. This tumor may also show clear cell, oncocytic, or columnar cells. This is the tumor type in which grading has the most prognostic and therapeutic impact. Overall 5-year survival for MEC ranges from 92% to 100% for low-grade tumors, 62% to 92% for intermediate-grade tumors, and 0% to 43% for high-grade tumors.[12] A few recent studies have also highlighted the value of grade in the management of patients.[13,14] Low-grade tumors generally require only surgical treatment, whereas high-grade tumors require adjuvant radiation and neck dissection. The management of intermediate-grade tumors is, however, controversial, and as shown below, the basis for this controversy lies in the method of grading.

In the initial description of MEC, the 2 subtypes of MEC included a "benign" and "malignant" version which equates today to low and high grade, respectively. It was only a matter of time before the need for an intermediate-grade category was recognized.[15] Current practice is to stratify tumors into low, intermediate, and high grade. The American Forces Institute of Pathology (AFIP) grading system,[16] Brandwein system,[17] and to a lesser extent, the modified Healey system[18] are the most popular systems used (Table 3). All categories assess a similar set of parameters, both cytomorphologic and architectural, and may also include perineural and angiolymphatic invasion. Both the AFIP and Brandwein system are point based, assigning point values to each adverse histologic parameters and with ascending point scores equating to a higher grade. The modified Healey system can be considered a "best-fit" type system: certain histologic parameters characterize a particular grade, and a tumor is graded based on its

TABLE 2. Risk Stratification of World Health Organization[2] Recognized Salivary Gland Malignancies

Low Risk	High Risk
Acinic cell carcinoma	Sebaceous carcinoma and lymphadenocarcinoma
Low grade mucoepidermoid carcinoma*	High grade mucoepidermoid carcinoma*
Epithelial-myoepithelial carcinoma	Adenoid cystic carcinoma†
Polymorphous low-grade adenocarcinoma	Mucinous adenocarcinoma
Clear cell carcinoma	Squamous cell carcinoma
Basal cell adenocarcinoma	Small cell carcinoma
Low-grade salivary duct carcinoma (low-grade cribriform cystadenocarcinoma)	Large cell carcinoma
Myoepithelial carcinoma	Lymphoepithelial carcinoma
Oncocytic carcinoma	Metastasizing pleomorphic adenoma
Carcinoma ex pleomorphic adenoma (intracapsular/minimally invasive or with low-grade histology)	Carcinoma ex pleomorphic adenoma (widely invasive or high-grade histology)
Sialoblastoma	Carcinosarcoma
Adenocarcinoma NOS and cystadenocarcinoma, low grade*	Adenocarcinoma and cystadenocarcinoma, NOS, high grade*

*Intermediate-grade variants of these tumors are controversial in the assignment of risk. For mucoepidermoid carcinoma this may depend on grading scheme used. For adenocarcinoma NOS, there is little data, but what is present suggests that intermediate grade should be placed in the high-risk group.
†Adenoid cystic carcinomas are all considered high risk in terms of local recurrence, but only solid adenoid cystic carcinoma (i.e. high pattern grade) is considered high risk for nodal metastasis.
NOS indicates not otherwise specified.

TABLE 3. Comparison of Grading Systems for Mucoepidermoid Carcinoma

Modified Healey[18] Qualitative	AFIP[16] Point Based	Brandwein[17] Point Based
Low grade Macrocysts, microcysts, transition with excretory ducts Differentiated mucin-producing epidermoid cells, often in a 1:1 ratio; minimal to moderate intermediate cell population Daughter cyst proliferation from large cysts Minimal to absent pleomorphism, rare mitoses Broad-front, often circumscribed invasion Pools of extravasated mucin with stromal reaction	Intracystic component < 20% = 2 pts	Intracystic component < 25% = 2 pts
	Neural invasion present = 2 pts Necrosis present = 3 pts	Tumor invades in small nests and islands = 2 pts Pronounced nuclear atypia = 2 pts
Intermediate grade No macrocysts, few microcysts, solid nests of cells Large duct not conspicuous Slight-to-moderate pleomorphism, few mitoses, prominent nuclei and nucleoli Invasive quality, usually well defined and uncircumscribed Chronic inflammation at periphery, fibrosis separates nests of cells and groups of nests	Mitosis (4 or more per 10 HPF) = 3 pts	Lymphatic and/or vascular invasion = 3 pts
	Anaplasia = 4 pts	Bony invasion = 3 pts > 4mitoses/10 HPF = 3 pts
High grade No macrocysts, predominantly solid but may be nearly all glandular Cell constituents range from poorly differentiated to recognizable epidermoid and intermediate to ductal type adenocarcinoma Considerable pleomorphism, easily found mitoses Unquestionable soft tissue, perineural and intravascular invasion Chronic inflammation less prominent, desmoplasia of stroma may outline invasive clusters		Perineural spread = 3 pts Necrosis = 3 pts
	Low grade = 0-4 pts Intermediate grade = 5-6 pts High grade = 7-14 pts	Low grade = 0 pts Intermediate grade = 2-3 pts High grade = 4 or more pts

AFIP indicates American Forces Institute of Pathology; HPF, high-power field; pts, points.

predominant morphologic features. Figures 1 and 2 illustrate tumors that would be considered low and high grade, respectively, under any grading system.

Despite the long-standing availability of these grading schema, many pathologists still avoid using them, mainly because of their cumbersome nature and the ambiguity of each of the histologic criteria.[4] In fact, in some cases, this lack of "user friendliness" is not necessary. For instance, the Brandwein grading scheme is point based, however, on close examination it is readily apparent that this grading scheme can easily be summarized as follows: there is a pool of intuitively adverse parameters (ie, infiltrative border, solid growth, perineural invasion, etc). A tumor with no adverse parameters is low grade, those with 1 bad parameter are intermediate grade, and those with 2 or more bad parameters are high grade.[19] Using this simple algorithm will result in exactly the same grade as the Brandwein system, and far more quickly. Modifications such as these to streamline existing grading schema are required to gain acceptability in the pathology community. It is important for standardized grading to be accepted, as evidence suggests that the reproducibility of any of these grading schemes, despite their flaws, is better than intuitive grading.[10,17]

A consensus among pathologists is clearly needed with regard to which MEC grading system is to be used, but level of reproducibility and awkwardness is only relevant if there is prognostic value to a grading system. In the broad sense, all grading systems are prognostically effective. However, each grading system varies with respect to the behavior of each particular grade. The AFIP system seems to "downgrade" tumors whereas the Brandwein system seems to "upgrade" tumors.[10] The danger of the AFIP system is the concern that some biologically aggressive tumors can be assigned a low-grade category and yet have a higher failure rate in treatment than expected. Conversely, the Brandwein system may categorize some indolent tumors as high grade, perhaps resulting in unnecessary radiation or additional surgery.

The management of intermediate-grade tumors is controversial as the behavior of intermediate-grade tumors varies dramatically in the literature. Astute observers will note that one main source of variation is the grading system used. For instance, Aro et al[13] using the AFIP system suggest that intermediate grade MEC cluster with high grade MEC, and should be treated in a similar fashion. However, Nance et al[14] showed that intermediate grade MEC cluster with low grade MEC using the Brandwein system. As a histologic correlate, the morphologic spectrum of intermediate-grade tumors varies depending on grading system (Figs. 3, 4).

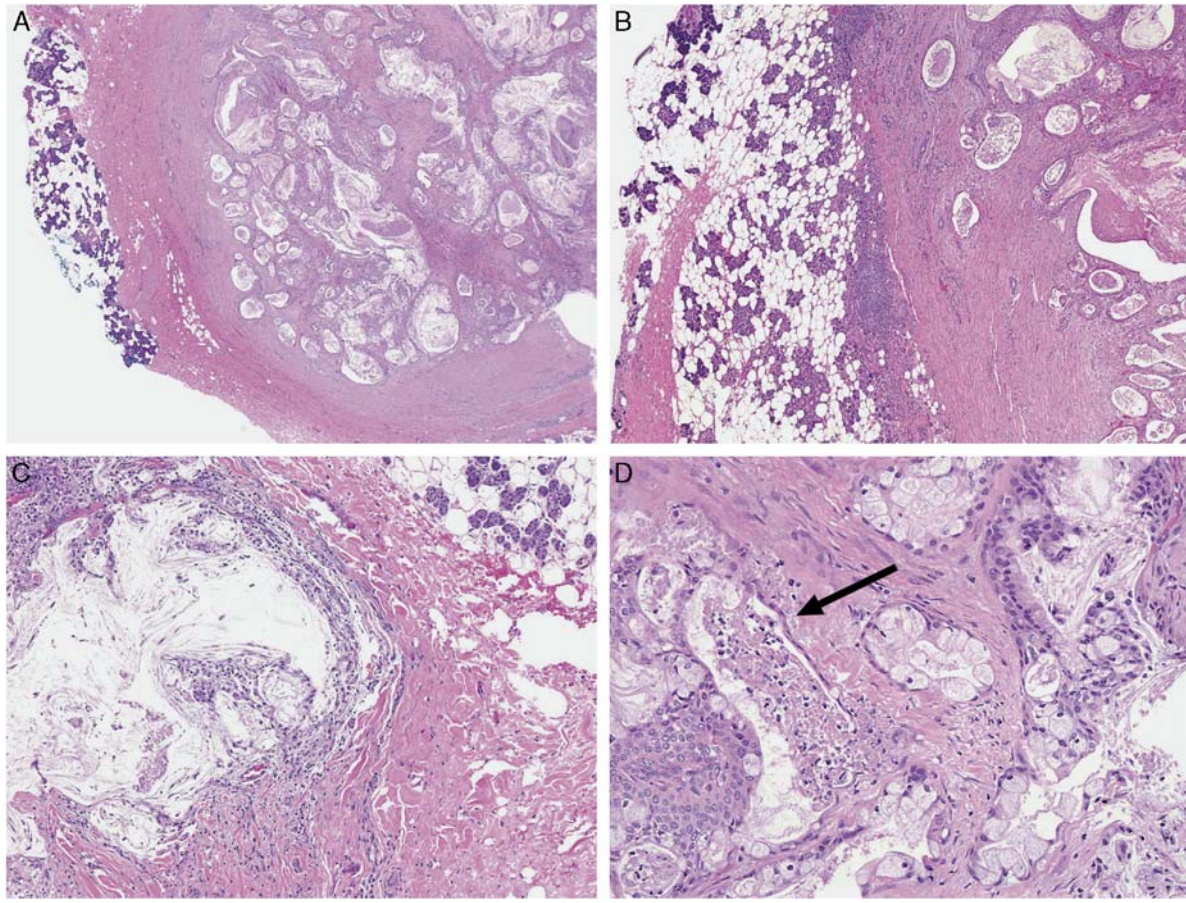

FIGURE 1. A parotid tumor that would be universally regarded as a low grade mucoepidermoid carcinoma. A, On scanning magnification, this tumor is well demarcated and comprised exclusively of cysts (H&E, 20×). B, The stroma is sclerotic and often surrounded by a lymphoid cuff (H&E, 40×). C, Cyst rupture and mucus extravasation are common (100×). D, There is an abundant mucous cell constituent that lines the inner layer of the cystic spaces. Immediately below this lining, tufts of intermediate and epidermoid cells are noted (left). Often, neutrophils and debris may be noted within cystic spaces (arrow), but this should not be construed as true tumor necrosis (H&E, 200×). H&E indicates hematoxylin and eosin.

The utility of grading as it applies to variants of MEC (ie, oncocytic, sclerosing, etc) is not clear. Limited evidence to date suggests that even oncocytic MECs that are considered high grade (Fig. 5) by a conventional grading scheme may behave indolently, with only 1 recurrence noted.[20] However, evidence is still insufficient to make a firm recommendation to discard grading for these variants.

One final issue regarding grading of MEC, particularly with respect to high-grade tumors, is the purity of MEC diagnosis itself. Historically, the term high grade MEC has been used interchangeably with adenosquamous carcinoma.[21] However, these tumors belong in separate categories. Although high grade MEC is a salivary gland tumor, adenosquamous carcinoma in the head and neck region is skin or surface mucosa derived, and is essentially a variant of squamous cell carcinoma that exhibits divergent glandular differentiation. Generally, adenosquamous carcinomas are very aggressive tumors, perhaps even more so than conventional squamous cell carcinoma.[22] Thus, the historic inclusion of these tumors in the MEC category has likely distorted our understanding of the biologic behavior of high grade MEC. Currently, there are specific criteria to distinguish adenosquamous carcinoma from MEC: the presence of surface dysplasia, more than focal keratinization, and discrete adenocarcinomatous foci.[23] A highly infiltrative growth pattern, and severe anaplasia are also typical of adenosquamous carcinoma, however, high grade MEC may occasionally show these features, hence these latter 2 are not absolute criteria for distinction.[19,23] As our understanding of MEC is largely based on retrospective cohorts, the potential for inclusion of adenosquamous carcinomas exists. In our experience, almost one fourth of cases that were called MEC in our files were reclassified as adenosquamous carcinomas using modern criteria.[19] The implication is that high grade MEC, although still an aggressive and potentially lethal tumor, may not behave as poorly as suggested, particularly by older literature.

Given the flaws in the current approach to grading, additional markers are desirable. Immunohistochemical staining for ki-67 has been fairly well-studied with respect to MEC.[24–26] Initial studies indicated that a proliferation index greater than 10% correlated with aggressive behavior. However, more recently, a multivariate analysis failed to show that ki-67 was an independently significant prognosticator. The utility of p53 immunostaining varies between studies. Some evidence suggests that p53 immunoexpression correlates with high histologic grade and even adverse outcome in a univariate fashion, but not in a

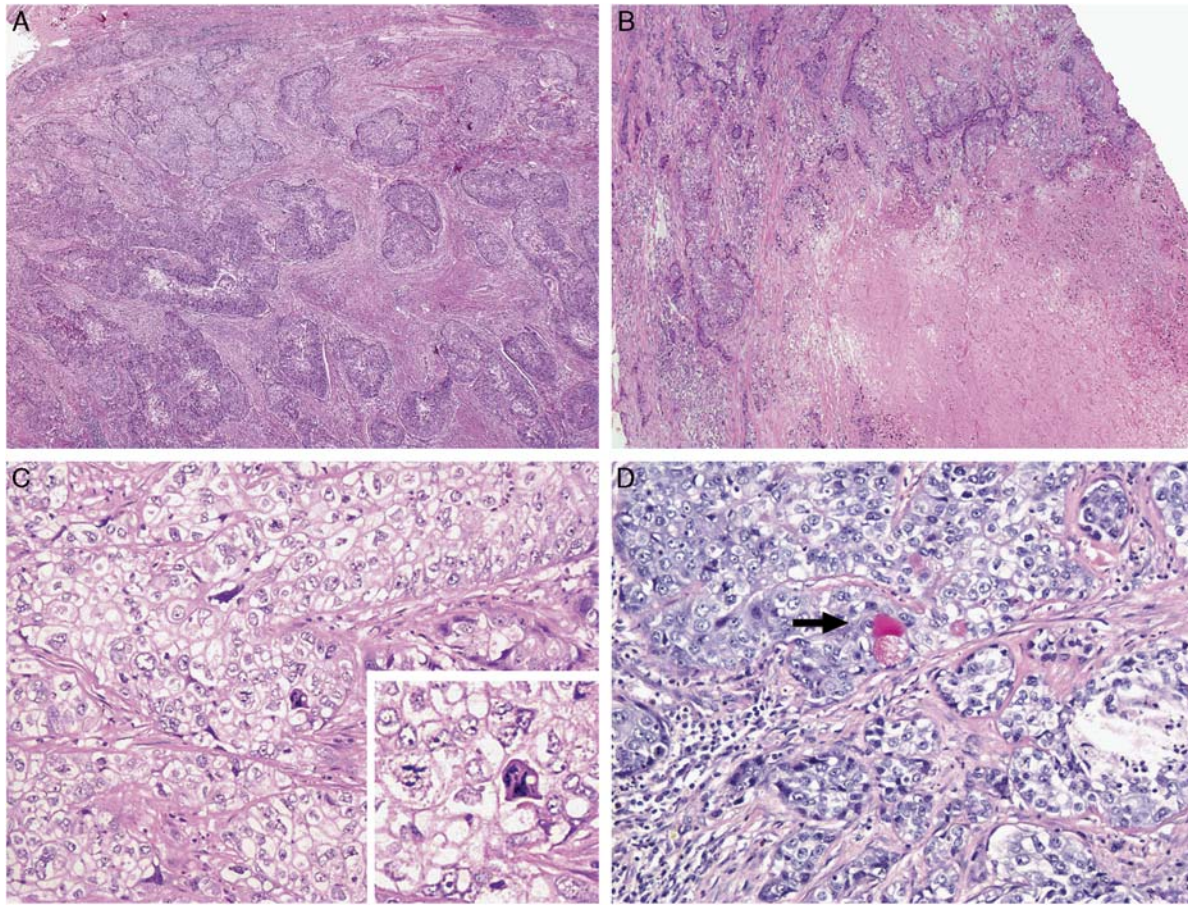

FIGURE 2. A parotid tumor that would be considered a high grade mucoepidermoid carcinoma in all grading systems. A, On scanning magnification, this tumor is entirely solid and not particularly well demarcated with nests percolating through sclerotic stroma (H&E, 20×). B, Large areas of necrosis are noted (H&E, 40×). C, Clear cell change is noted, but also accompanied by pronounced nuclear atypia (H&E, 200×). Inset: Two mitoses noted in the same high-power field. D, A mucicarmine stain highlights rare single mucous cells (arrow) within a confluent growth of epidermoid cells (200×). H&E indicates hematoxylin and eosin.

multivariate fashion.[27,28] Of note, p53 immunoexpression may not correlate with *TP53* mutation status, and mutations have been reported even in low-grade tumors.[29,30] Other markers such as epidermal growth factor receptor (EGFR), extracellular-signal-regulated kinases, and human epidermal growth factor receptor 2 (HER-2) have been noted to correlate with grade and in some cases, outcome in a univariate manner, but have not been validated as independent prognosticators.[31,32]

Perhaps the most promising molecular prognostic marker to date in MEC is the t(11;19)(q21; p13) which results in the fusion of the *MECT1* (also known as *CRTC1, TORC1*, and *WAMTP1*) gene at 19p13 and *MAML2* gene at 11q21. The presence of the translocation in MEC ranges from 38% to 82% in published series, and has a predilection for low and intermediate-grade tumors (Fig. 6). In addition to this correlation, the translocation is also a favorable prognostic parameter. Okabe et al[33] have even demonstrated that *MECT1-MAML2* status is an independent prognosticator. However, the prevalence of the translocation within high-grade tumors varies tremendously. Earlier studies show no evidence of rearrangement in high grade MEC,[33,34] but more recent studies show that this is a frequent event even within the high-grade category.[19,35] To some extent the variability may represent differences in grading criteria (ie, some Brandwein high-grade tumors would be considered intermediate-grade tumors under the AFIP scheme), however, this higher prevalence of the *MECT1-MAML2* translocation in recent studies of high grade MEC may also represent a refinement of the actual diagnosis—perhaps earlier series included tumors frequently misdiagnosed as high grade MEC, namely adenosquamous carcinoma. Even within the high grade MEC subgroup, translocation status has a tendency to identify more indolent tumors.[19] However, *MECT1-MAML2* positivity does not ensure indolent behavior, as even a fraction of these tumors may prove lethal.[19,36] Anzick et al[36] have shown that deletions in the *CDKN2A* region may predict an aggressive behavior even in *MECT1-MAML2*-positive tumors. Recently, a variant location, CTRC3-MAML2 has been described and characterized as a similarly favorable prognosticator, albeit in a younger age group.[37]

Adenoid Cystic Carcinoma

Even dating back as early as 1853, the distinctive appearance of the tumor that is now known as ACC was noted by Robin and Laboulbene.[38] ACC is comprised of tumor cells with scant cytoplasm and hyperchromatic nuclei, imparting an intensely "blue" or basaloid appearance

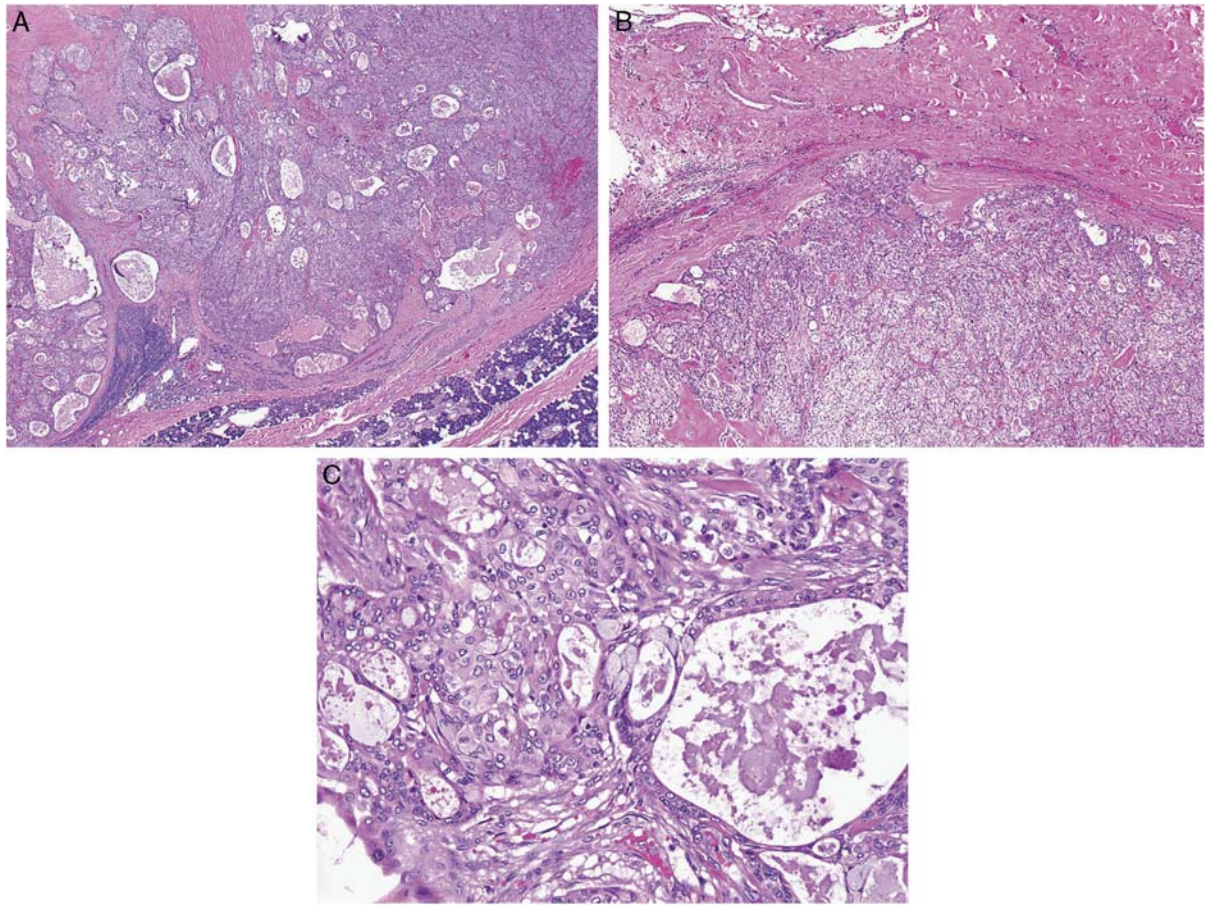

FIGURE 3. A tumor that may be considered an intermediate grade mucoepidermoid carcinoma. A, This is a predominantly solid but well-demarcated tumor with scattered microcysts (H&E, 20×). This would qualify for 2 points under both the AFIP and Brandwein grading scheme. B, On closer examination, focal border irregularities are noted though this tumor is not frankly infiltrative (H&E, 40×). C, Mucous cells are still readily identifiable though they comprise a smaller proportion of tumor cells. All cell types seen here are bland, monomorphic, and devoid of mitotic activity (H&E, 200×). Under the Brandwein grading scheme, this would qualify as an intermediate-grade tumor, but under the AFIP scheme this would still be considered low grade. AFIP indicates American Forces Institute of Pathology; H&E, hematoxylin and eosin.

at low-power magnification. In addition, characteristic is the prominent acellular myxohyaline matrix that forms "cylinders" within tumor nests. ACC is a biphasic tumor composed of ducts and basal/myoepithelial that can be arranged in a tubular, cribriform, or solid growth pattern. The biologic course of this tumor overall is slow but relentless—5-year survival is favorable at roughly 75% to 80%, but 15-year survival is poor at about 35%.[39,40] ACC has a fairly unique behavior among salivary gland malignancies. It is a tumor that is cytomorphologically bland and monomorphic, yet among the most infiltrative and permeative of carcinomas. As such it is extremely locally aggressive placing it in a high-risk category with regard to the use of adjuvant radiation. In contrast, ACC in conventional form does not seem to have much risk of lymphatic spread as regional lymph nodes are involved in only about 5% of case. Thus, many institutions may not perform neck dissections routinely on ACC patients.[3]

A quick and simple form of grading of ACC relying solely on growth pattern has evolved and been shown to be prognostically useful in several series.[40–42] As early as 1958, Patey and Thackray[43] noted that a solid growth pattern imparts a poor prognosis. Subsequently, grading of this tumor has evolved into stratification into 3 grades of increasing aggressiveness based on predominant growth pattern[41,42]: grade 1: tubular, grade 2: cribriform, and grade 3: solid (Fig. 7). Generally, a tumor with a greater than 30% solid component belongs in the "grade 3" category. However, it is suggested that any solid component imparts a poor prognosis, and that the relationship between solid growth pattern percentage and prognosis is somewhat linear and that assigning a cutoff may be arbitrary.[42]

The current WHO classification refers to tumors by predominant pattern rather than actually assigning a numeric grade, likely for a few reasons.

First, one issue with pattern grade in ACC is its prognostic utility independent of tumor stage. Several studies indicate that grade does correlate with prognosis, but typically, the independence of grade from stage had not been evaluated.[40–42] Spiro and Huvos[39] suggest that grade does not provide an prognostic benefit beyond correlation with stage, however, da Cruz Perez et al[44] show that grade is an independent prognosticator on multivariate analysis. One potential difference is that the grading scheme used by

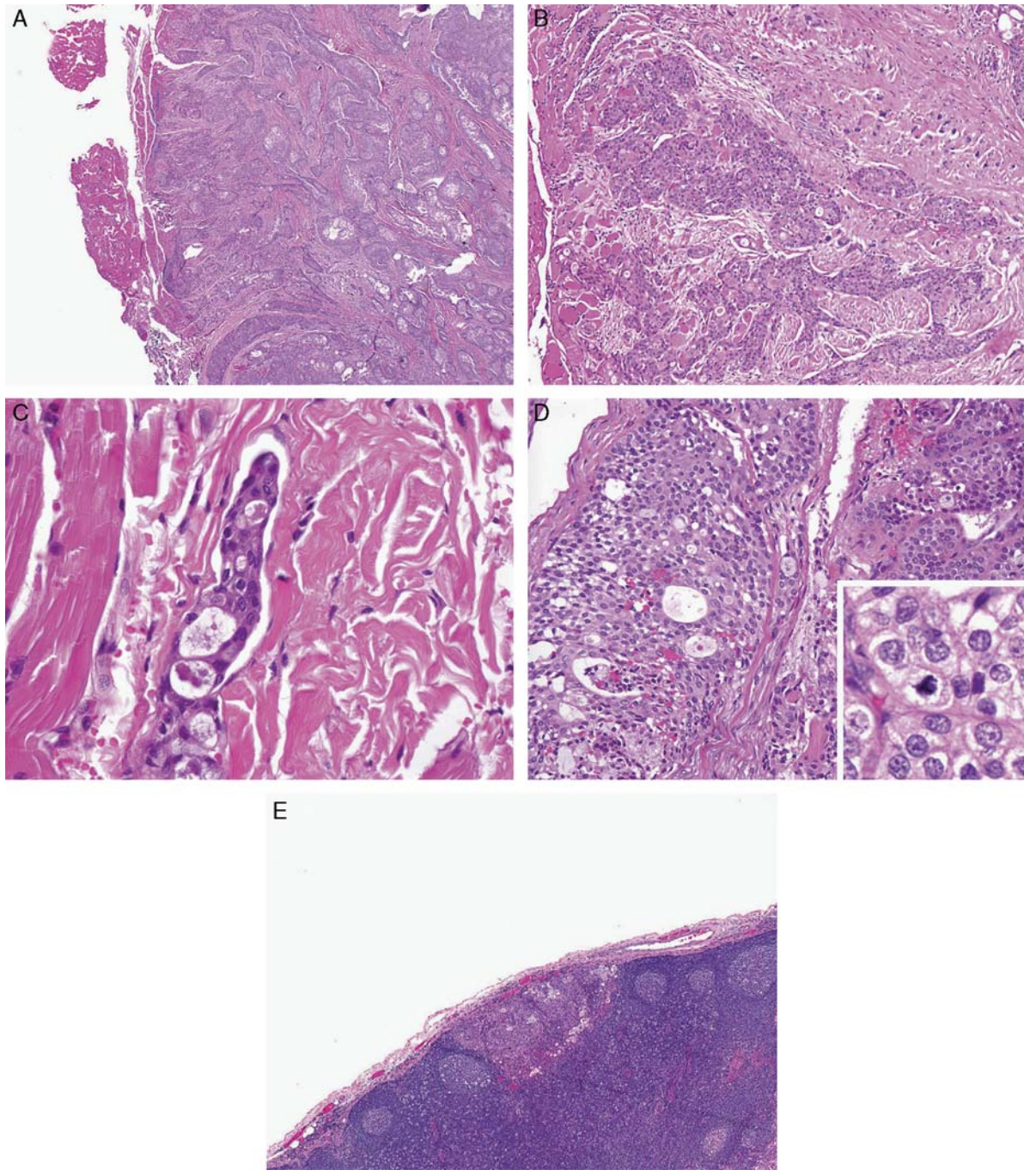

FIGURE 4. Another tumor that may be considered an intermediate grade mucoepidermoid carcinoma. A, This is also a solid tumor that appears well demarcated at this magnification (H&E, 20×). B, However, on closer examination, this tumor appears quite infiltrative permeating skeletal muscle (H&E, 40×). C, Vascular invasion is present, although perineural invasion is not noted (H&E, 400×). D, Cytologically, the tumor is comprised mainly of intermediate and epidermoid cells, although mucous cells are easily noted (H&E, 200×). This tumor is monomorphic and fairly bland, though mitotic activity is increased (inset; H&E, 400×). This tumor achieves 7 points under the Brandwein scheme and easily attains high-grade status. However, under the AFIP system, vascular invasion and tumor infiltration as small nests are not counted. With the solid growth and mitotic activity (6 points), this would still be considered intermediate grade using AFIP criteria. E, This patient had cervical lymph node metastases (H&E, 100×). AFIP indicates American Forces Institute of Pathology; H&E, hematoxylin and eosin.

Spiro and Huvos[39] differs from the typical scheme particularly with regards to a solid component cutoff of greater than 50%. The difficulties in conversion between the 2 grading schemes are illustrated in Table 4. For example, tumor that barely qualifies as pattern grade 3 under the Perzin et al[41]/Szanto et al[42] scheme (ie, little over 30% solid

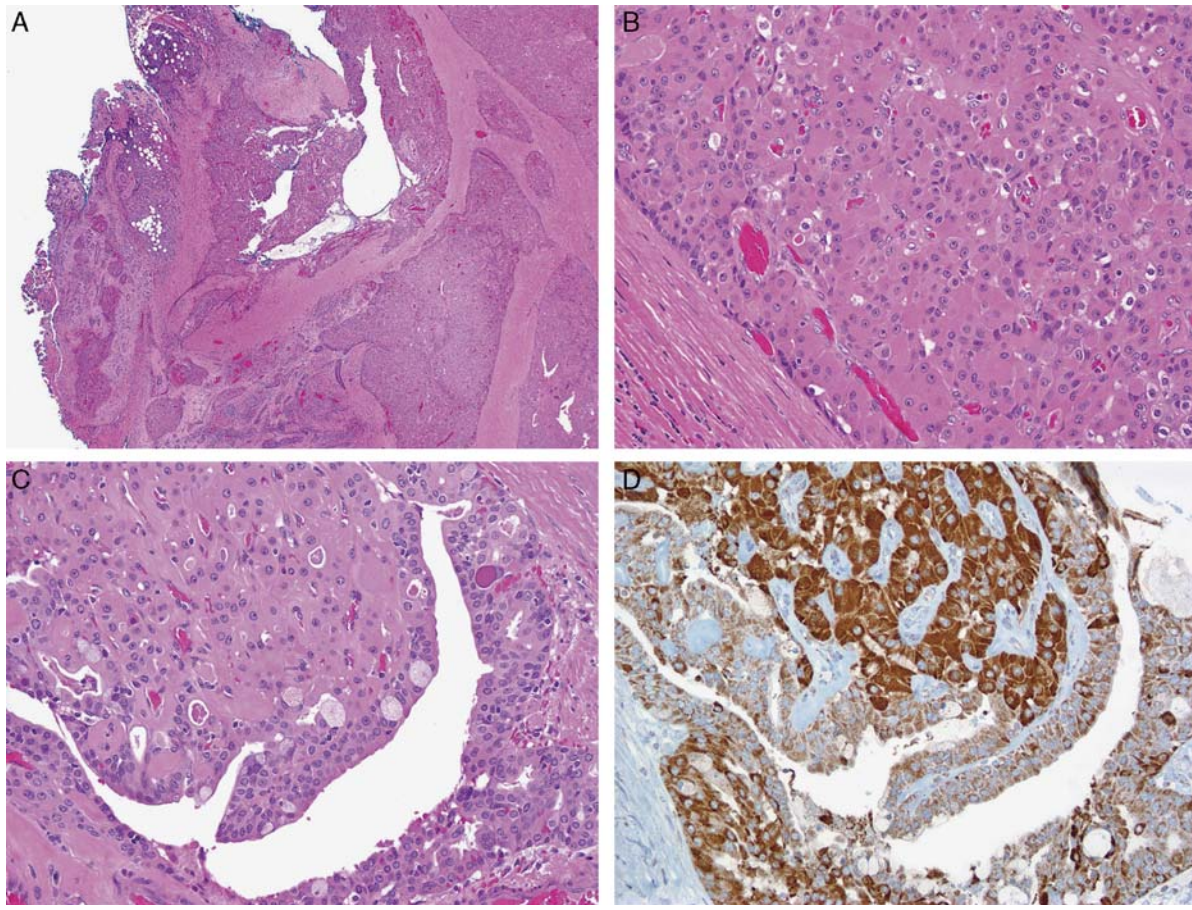

FIGURE 5. Oncocytic MEC. A, This variant of MEC is often solid, and, in this particular case, infiltrative qualifying as intermediate or high grade depending on grading system (H&E, 20×). B, Most of the tumor is comprised of solid nests of oncocytic cells that likely represent modified intermediate cells (H&E, 200×). C, However, areas within the tumor show cystic change and more conventional-appearing foci with less oncocytic epidermoid/intermediate cells as well as mucous cells (H&E, 200×). D, An antimitochondrial antibody stain highlights stronger staining in the oncocytes as compared with the more conventional-appearing region (200 ×). H&E indicates hematoxylin and eosin; MEC, mucoepidermoid carcinoma.

growth), would still be pattern "grade 1" under the Spiro and Huvos[39] grading scheme, a 2 grade discrepancy! Another study by Prokopakis et al failed to show prognostic utility for grade even with the Perzin et al[41]/Szanto et al[42] scheme, however, this study does not seem to have included a pathologist co-investigator, which essentially invalidates any assertions made by this group on histologic grade.[45] Reproducibility of either pattern-based scheme is not well addressed in the literature. In 1 study,[46] however, the scheme used by Spiro and Huvos[39] has less interobserver variability.

However, perhaps more importantly, ACC grading is not a subject of heated debate because it is not particularly useful in patient management. Regardless of pattern, all ACC are treated with surgery plus irradiation because of their locally aggressive and infiltrative nature. With regard to the neck, most decisions on the neck dissection will not depend on grade, even though there is some evidence to suggest that solid/grade 3 ACCs have a higher likelihood of lymph node metastasis.[3]

Despite being among the oldest recognized salivary gland carcinomas, a molecular understanding of ACC is still fairly rudimentary; let alone the relevance of molecular alterations to prognosis. There is some evidence to suggest that p53 alterations correlate with a more aggressive behavior,[44,47,48] although independent prognostic value was only shown in 1 series.[44] Cyclin D1 and C-kit have been biomarkers of interest in ACC, however, immunoexpression of these markers seems to be too ubiquitous to be prognostically useful. Of note, amplification of the *CCND1* gene,[49] and activating *KIT* mutations[50] are rare. Chromosomal alterations have been studied in some detail using methodologies ranging from conventional karyotype to array comparative genomic hybridization. Figure 8 summarizes the common chromosomal alterations in 153 ACC characterized in the literature.[51–60] The most common chromosomal losses involve 1p, 6q, and 12q, and more specifically involve the regions, 1p32-p36, 6q23-q27, and 12q12-q14. The most common chromosomal gains are seen in chromosome 8 and 22, more specifically in the regions, 8q24 and 22q13. As expected, Vekony et al[58] confirm that a high number of chromosomal alterations is an adverse prognosticator. More specifically, Rao et al[60] demonstrate that deletions in the 1p32-p36 region are predictive of aggressive behavior. However, although promising, it is still not clear whether these markers have prognostic value

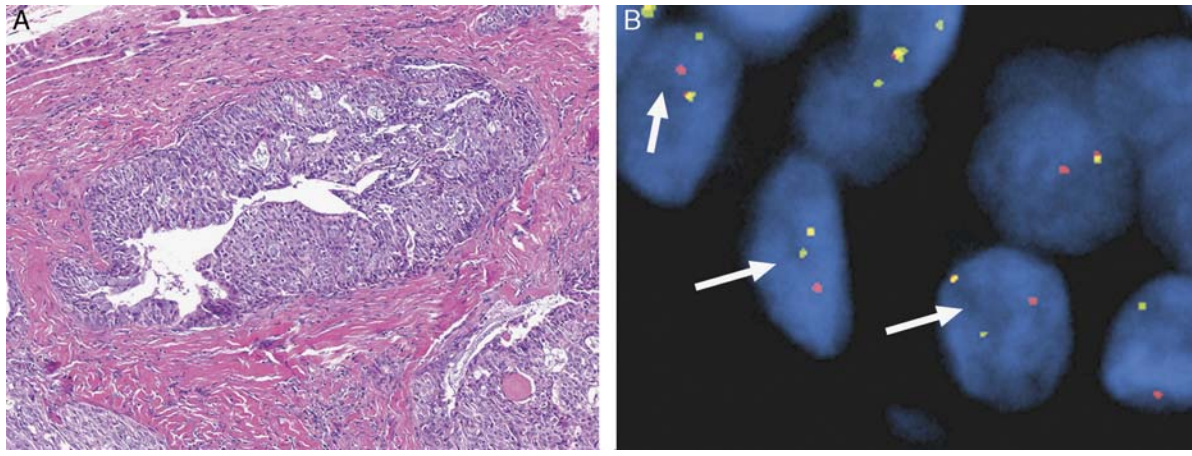

FIGURE 6. FISH for *MAML2* rearrangement detection using breakapart probes. A, Intermediate grade mucoepidermoid carcinoma (hematoxylin and eosin, 100×). B, By FISH, the cells (arrows) demonstrate 1 intact MAML2 copy indicated by juxtaposition of the fluoroisothiocyanate (green) and specrum orange (red)-labeled probes (yellow signal), and 1 split copy resulting in the separation of the red and green signals within the cell. FISH indicates fluorescence in-situ hybridization.

independent of standard clinicopathologic parameters. A search for candidate genes within these frequently altered chromosomal regions that are implicated in ACC tumorigenesis may ultimately yield useful prognostic, and perhaps, even therapeutic markers. Recently, a frequent translocation t(6;9) which results in the fusion of *MYB* and *NFIB*

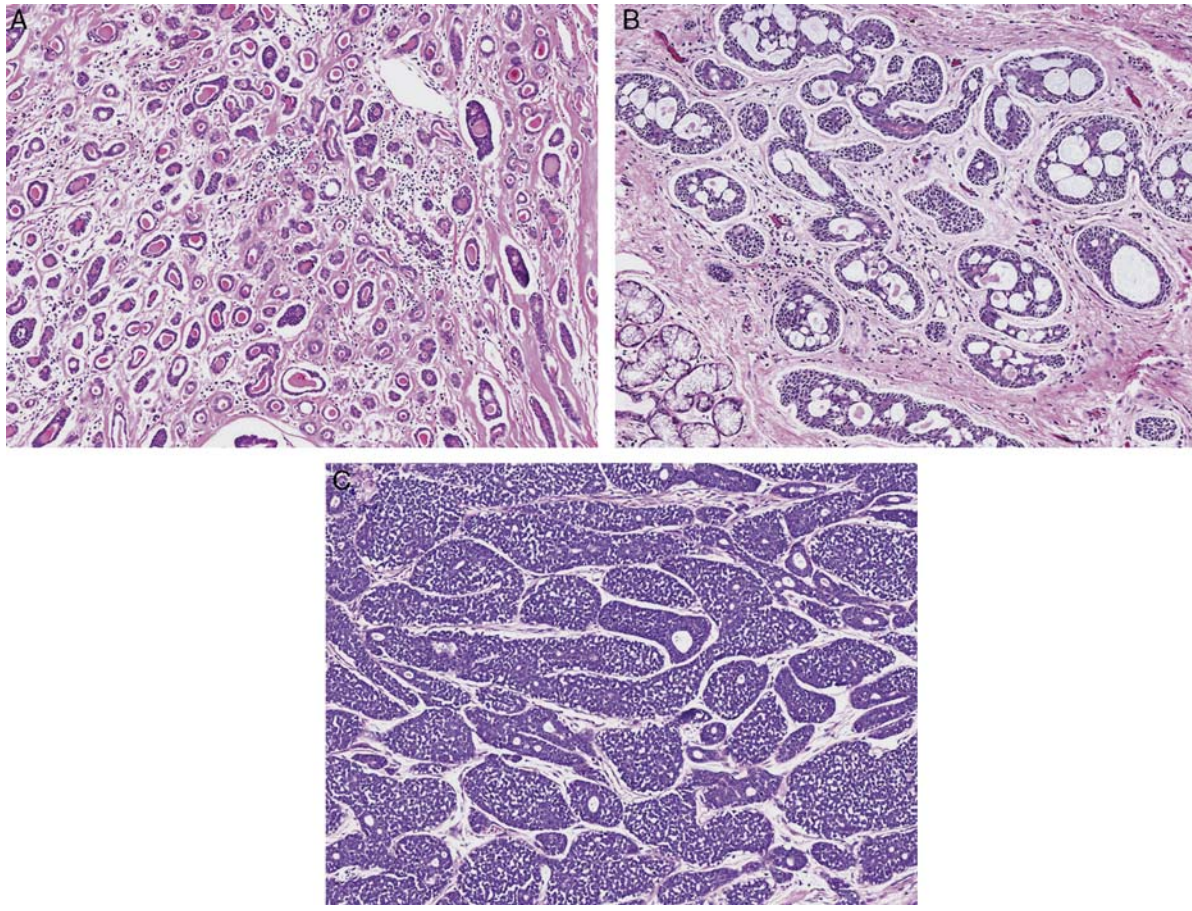

FIGURE 7. Patterns in adenoid cystic carcinoma. A, Tubular growth (H&E, 100×), (B) Cribriform growth (H&E, 200×), and (C) Solid growth (H&E, 100×). All growth patterns are characterized by a proliferation of small cells with scant cytoplasm, and angulated hyperchromatic but monomorphic nuclei. Stroma varies from myxoid to hyaline, and there is often "clefting" or retraction of tumor nests from surrounding stroma. H&E indicates hematoxylin and eosin.

TABLE 4. Comparison of Common Pattern Grading Schemes in Adenoid Cystic Carcinoma

Grade	Perzin et al[41] and Szanto et al[42]	Grade	Spiro and Huvos[39]
1	Predominantly tubular, no solid component	1	Mostly tubular or cribriform (no stipulations on minor solid components)
2	Predominantly cribriform, solid component < 30% acceptable		
3	Solid component > 30%		
		2	50% solid
		3	Mostly solid

has been characterized.[61] However, although about 1/4 of primary ACC harbor this translocation, at this point there seems to be no prognostic relevance.

One variant of ACC, namely, ACC with high-grade transformation (ACC-HGT) deserves special mention. ACC-HGT is a rare, highly aggressive variant of ACC (Fig. 9). Although conventional ACC, regardless of pattern, is comprised of monomorphic fairly small angulated nuclei, ACC-HGT is characterized by areas of pleomorphic mitotically active high-grade carcinoma in juxtaposition with areas of conventional ACC of any pattern.[62] Conventional ACC is a biphasic tumor with ducts and myoepithelial cells, but the transformed component in ACC-HGT is purely of a ductal phenotype with a solid or cribriform appearance. Transformed components show prominent nuclear size and chromatin variability. Common features include fibrocellular desmoplasia, abundant mitoses, necrosis, and microcalcifications. Unique patterns in HGT include micropapillary and squamoid growth. However, there is still morphologic overlap between solid conventional ACC and ACC-HGT, and when seen together, the transition from solid conventional ACC and ACC-HGT is often gradual. Table 5 delineates key distinguishing features between solid conventional ACC and ACC-HGT. Basically, the aggressive nuclear, stromal, architectural, and immunohistochemical features common to both variants are more exaggerated in HGT, whereas solid conventional ACC shows only slight deviation from tubular or cribriform conventional ACC.

About 40% of tumors have p53 alterations suggesting that this has a role in HGT.[62,63] Furthermore, recent array comparative genomic hybridization studies have implicated gains in chromosomal regions: 8q24, 17q11.2-q12, 17q23, and 15q11-13. The chromosomal region 8q24 is of interest as it harbors the *C-MYC* gene locus and even in conventional ACC, it is the region of most frequent gains. These tumors have an exceptionally poor prognosis with a median survival ranging from 12 to 36 months, and may thus be even more aggressive than solid or grade 3 ACC. Unlike conventional ACC, this tumor has a lymph node metastatic rate of over 50%. Thus, if a transformed

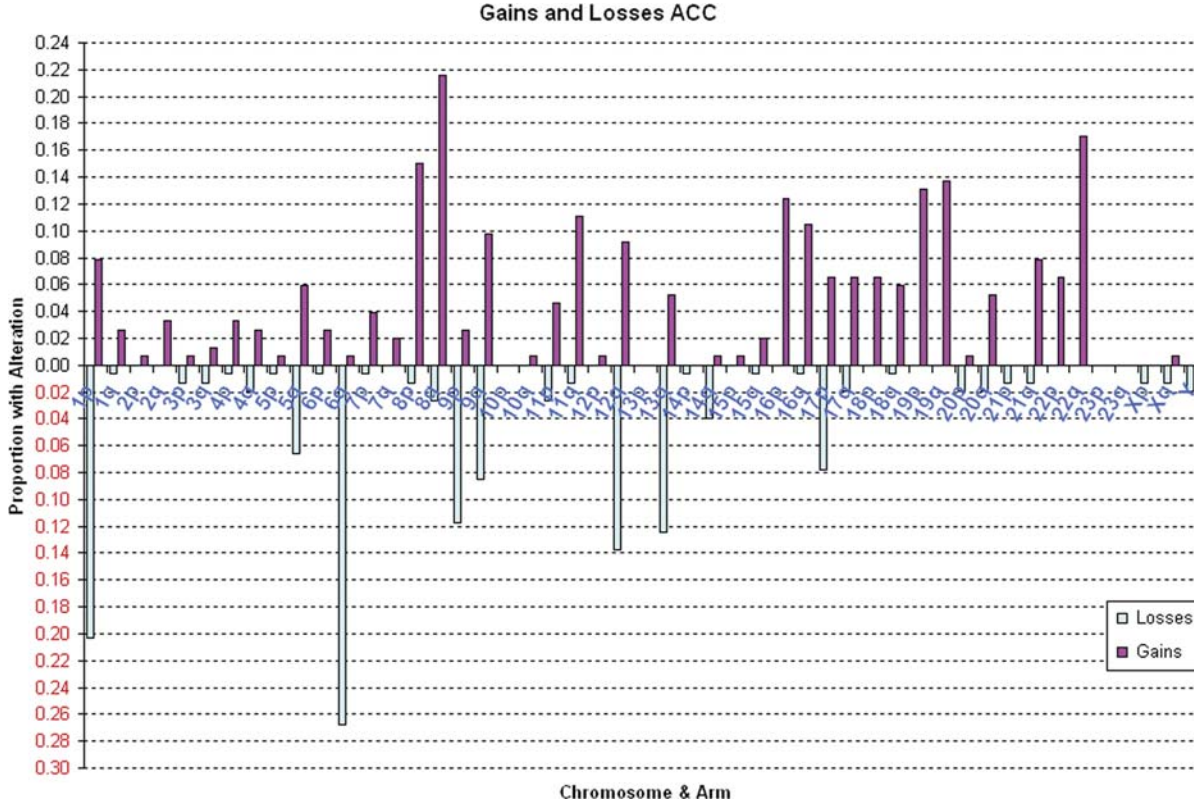

FIGURE 8. Chromosomal gains and losses in adenoid cystic carcinoma (ACC). The most common chromosomal losses involve 1p, 6q, and 12q, and more specifically involve the regions, 1p32-p36, 6q23-q27, and 12q12-q14. The most common chromosomal gains are seen in chromosome 8 and 22, more specifically in the regions, 8q24 and 22q13.

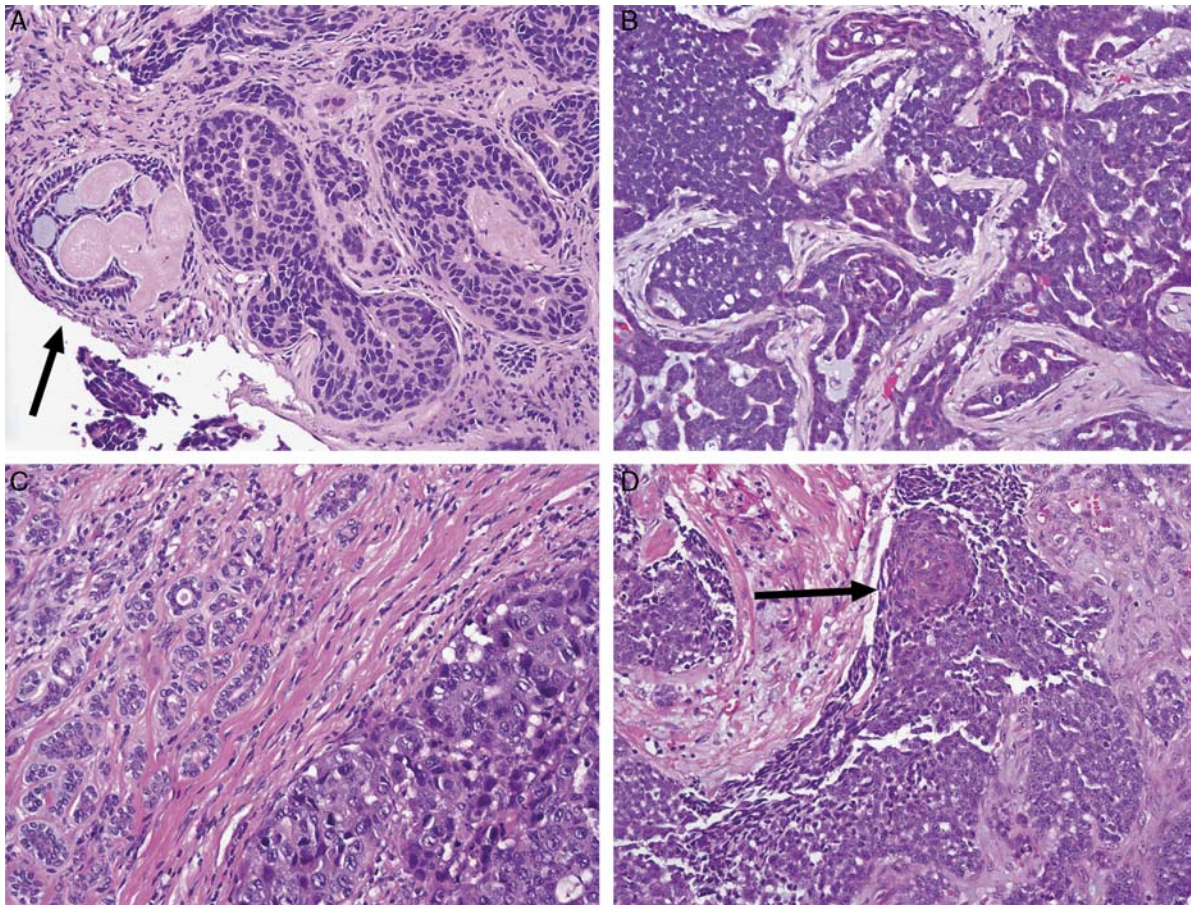

FIGURE 9. Various morphologic patterns in ACC-HGT. A, The transformed component may have a tubulocribriform architecture although it would be comprised of highly pleomorphic cells of a ductal phenotype in contrast to the small angulated biphasic morphology seen in the conventional component (arrow) (H&E, 200×). B, Often, the transition from conventional ACC to the transformed component may be gradual with both components blending imperceptibly. Here, the solid conventional component (left) transitions subtly even within individual tumor nests into a micropapillary high-grade adenocarcinoma. Note the change in cytoplasmic tinctorial quality (H&E, 200×). C, However, the classic ACC-HGT, earlier known as "dedifferentiated ACC" consists of a tubular or cribriform conventional component (left) with and abrupt transition to an undifferentiated carcinoma (H&E, 200×). D, Rarely squamoid change may be noted (arrow) which can be difficult to distinguish from basaloid squamous cell carcinoma (H&E, 200×). ACC-HGT indicates adenoid cystic carcinoma with high-grade transformation; H&E, hematoxylin and eosin.

component is found in an ACC, a neck dissection is likely warranted.

Carcinoma ex Pleomorphic Adenoma

Carcinoma ex pleomorphic adenoma (CAxPA) comprises 10% to 15% of all salivary gland malignancies and is prevalent in the sixth decade, roughly 10 years after the mean age at which patients with pleomorphic adenoma present. There is a slight female predilection, although this varies between different studies. Although the parotid gland is the most common site for CAxPA (approximately 75%), this tumor category can potentially arise in any site where pleomorphic adenomas occur. Many cases present de novo, but roughly 7% to 16% represent malignant transformation of a recurrent pleomorphic adenoma.[64] CAxPA is a class of lesions that is often viewed as automatically high risk, particularly in clinical circles.[1,3,6] In general, this holds true with a 5-year survival of as low as 37%, locoregional recurrence in 55%, and distant metastases in up to 42% of patients.[64]

Similarly, the carcinomatous components are typically high grade or of an aggressive histologic type, with high-grade adenocarcinoma, not otherwise specified, and salivary duct carcinoma being the most common histologic subtypes of the carcinomatous component (Fig. 10). However, as many as 15% of tumors are low grade and may behave in a more indolent manner (Fig. 11).[64,65] Interestingly, with regard to histologic subtype, a myoepithelial carcinomatous component has been correlated in a recent study with more aggressive behavior although it is unclear whether this is independent of the cytomorphologic grade.[65] Nonetheless, given the histologic and biologic diversity, it is clear that CAxPA is not actually a specific diagnosis, but rather a class of entities. As such, the carcinomatous component should be characterized as to type and grade. A rough quantitation of the carcinomatous component is recommended. Although there is no hard evidence that this is yet a useful prognosticator, a high percentage of carcinoma relative to pleomorphic adenoma is more typical of frankly or widely invasive CAxPA.[4,65]

More recently, the push has been to stratify CAxPA based on extent of invasion. Intracapsular CAxPA describes a

TABLE 5. Comparison of Solid Conventional ACC and High-grade Transformation

Features	Solid Conventional ACC	ACC With High-grade Transformation
Chromatin	Dark, homogeneous	Vesicular or heterogeneously dispersed
Nuclear membranes	Delicate	Thickened or irregular
Nucleoli	Present but indistinct	Prominent central
Nuclear size	**At most twice the size of grade I-II ACC nuclei. Uniform size distribution**	**At least 2-3 times the size of grade I-II ACC nuclei (typically more). At least 2-fold nuclear variation**
Cytoplasm	Scant to nearly absent	Scant to moderate
Growth	**Solid nests, rarely spanning more than a 40× HPF**	**Solid confluent nests to sheets often filling a 40× HPF**
Stroma	**Paucicellular myxoid or hyaline**	**Fibrocellular desmoplastic**
Comedonecrosis	Focally present, usually punctuate	Often present, punctuate to large zones
Microcalcifications	Rarely present	Often present
Unique features		**Micropapillae, squamoid areas**
Mitoses	Generally < 10/HPF	Usually > 10/HPF
Albuminal cell layer presence by immunohistochemistry	**Present and complete**	**Incomplete and at least focally absent**
Ki-67	< 50%	> 50%
p53 overexpression (strong reactivity in > 50% of cells)	**Rare**	**Common**

Bold characters represent major features.
ACC indicates adenoid cystic carcinoma; HPF, high-power field.
Adapted with permission from *Am J Surg Pathol*. 2007;31:1683–1694.

carcinomatous component that is confined to within a pleomorphic adenoma, whereas minimally invasive CAxPA describes a tumor with minimal extent beyond the capsule (Fig. 12). The WHO definition for minimal invasion is less than 1.5 mm of invasion beyond the capsule. Both these subgroups are considered indolent variants that should not be considered equivalent to the typical CAxPA. However, although initial studies have suggested that intracapsular and minimally invasive CAxPA have no risk of recurrence or lethality,[64,66] Katabi et al[65] indicated as many as 25% of their intracapsular or minimally invasive CAxPA behaved in an aggressive fashion. Given this conflicting data, the validity of this concept of these architecturally indolent variants of carcinoma needs to be further substantiated by additional studies.

As CAxPA is a heterogenous group of entities with a presumably unique pathogenesis depending on carcinoma type, it is difficult to apply a generic set of biomarkers and obtain enhanced prognostic utility beyond the parameters noted above. Indeed, p53 and c-erbB-2 immunoexpression have not been shown to have any prognostic value.[64,67] In contrast, Katori et al[68] suggest that a high Ki-67 proliferation index and EGFR overexpression may correlate with more aggressive behavior, although it is not clear whether this is independent of grade, tumor type, extent, and stage. Thus, ancillary markers currently have no practical utility in the management and prognosis of CAxPA.

Acinic Cell Carcinoma

AciCC was described by Nasse in 1892[69] as a benign tumor; it was only in 1953 that its malignant potential was realized with the description of 5 aggressive cases by Buxton et al.[70] Nonetheless, AciCC is generally has a favorable prognosis. According to a National Cancer Database review (1985 to 1995), the 5-year disease-specific survival for AciCC was shown to be 91% (n = 1353),[71] which is in keeping with large single institution or consultative series.[8,72,73] However, late recurrences, local or distant, may be noted several decades after initial diagnosis, and up to 25% may eventually die of disease.[8] The recurrence rate varies depending on series and ranges from roughly 10% to 45%. Metastases may be noted in 8% to 19% of patients. National Cancer Database data indicate a 10% metastatic rate to lymph nodes and a 3% metastatic rate to distant sites.[71]

AciCC is typically considered a low-grade carcinoma for management purposes. Although most AciCCs are indeed indolent, as noted above, there are a significant number of patients with unfavorable outcomes arguing against this generality. In fact, AciCCs would seem to be amenable to a grading system as indicated by the differential biologic behavior denoted above. In some series, various histologic parameters have been noted to correlate with adverse outcome in AciCC (Fig. 13). For instance, Lewis et al[8] have noted, that mitoses, atypia, and desmoplasia are among the histologic parameters that correlate with adverse outcome. Similarly, Gomez et al[73] noted that more than 2 mitoses per 10 high-power fields, atypical mitosis, vascular invasion, perineural invasion, pleomorphism, and necrosis are all adverse histologic parameters. Conversely, Michal et al[9] report that AciCCs that are surrounded by a prominent "encapsulated" lymphoid stroma behave more favorably. Grading schemes based on these parameters have been attempted. Batsakis et al[74] proposed a 3-tier grading scheme based on various adverse pathologic parameters, however, this scheme was fairly arbitrary without any prior or subsequent validation. Gomez et al[73] used a 2-tiered grading scheme, and designated tumors with greater than 2 mitoses per 10 high-power fields or necrosis as high grade and showed that this scheme did, in contrast, correlate with disease-free and overall survival. Thus, although no single grading scheme has been popularized for AciCC, it seems that assigning a grade is of prognostic value and may improve the management of patients by identifying subgroups that may need adjuvant therapy and/or neck dissections.

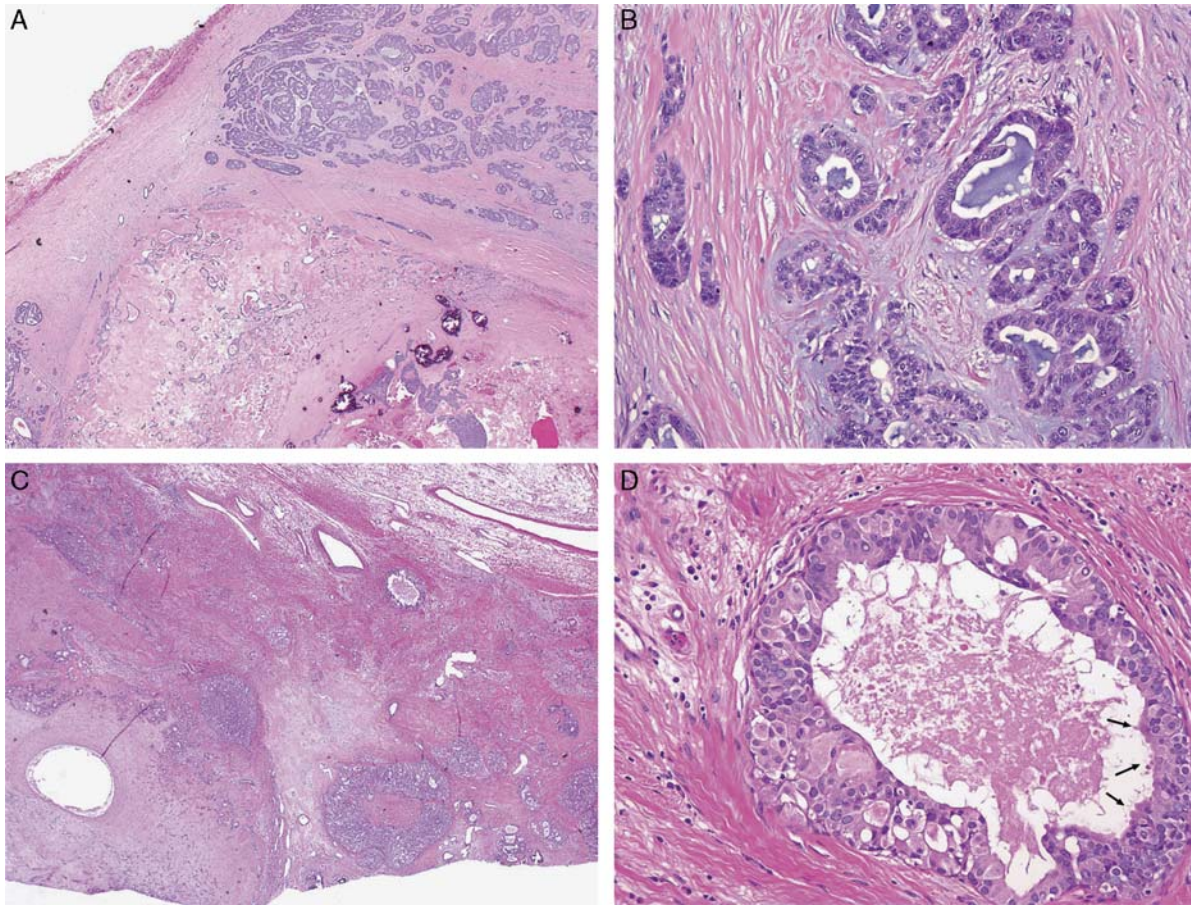

FIGURE 10. Common forms of CAxPA. A, The carcinomatous component is often aggressive, and often remains of a PA is rather small and consists of a hyalinized calcified nodule (bottom) (H&E, 20×). B, The carcinomatous component here is a high-grade adenocarcinoma, not otherwise specified. C, This CAxPA has more of a myxoid stroma in the PA component (bottom left) (H&E, 20×). D, The adenocarcinomatous component here shows abundant granular pink cytoplasm, cribriform growth, and apical snouts (arrows) indicative of apocrine morphology consistent with salivary duct carcinoma. CAxPA indicates carcinoma ex pleomorphic adenoma; H&E, hematoxylin and eosin.

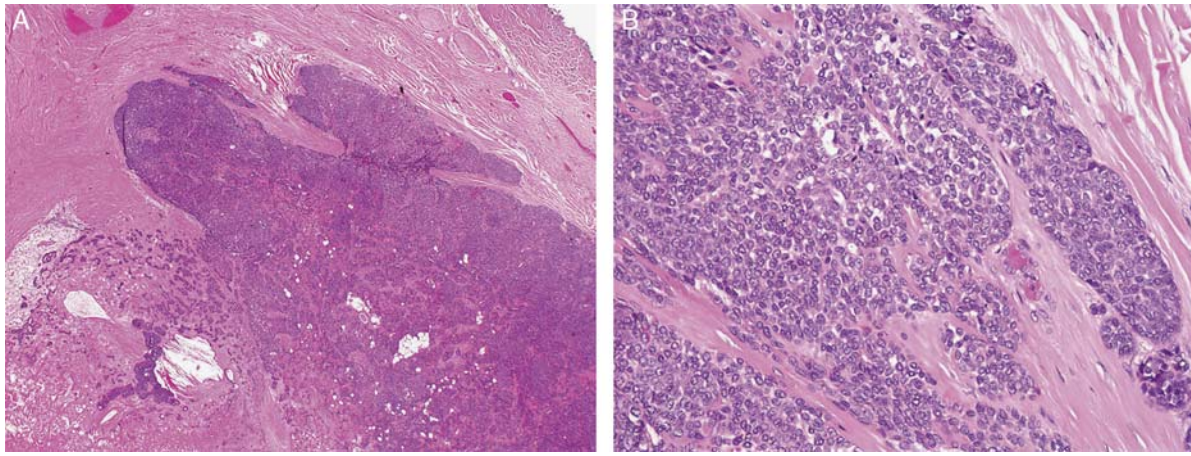

FIGURE 11. Low grade carcinoma ex pleomorphic adenoma. A, This tumor also consists mainly of carcinoma with a minor pleomorphic adenoma component (bottom left) (H&E, 20×). B, However, the carcinomatous component consists of a solid proliferation of bland, monomorphic cells with scant cytoplasm in a sclerotic stroma compatible with a (low grade) myoepithelial carcinoma (H&E, 200×). H&E indicates hematoxylin and eosin.

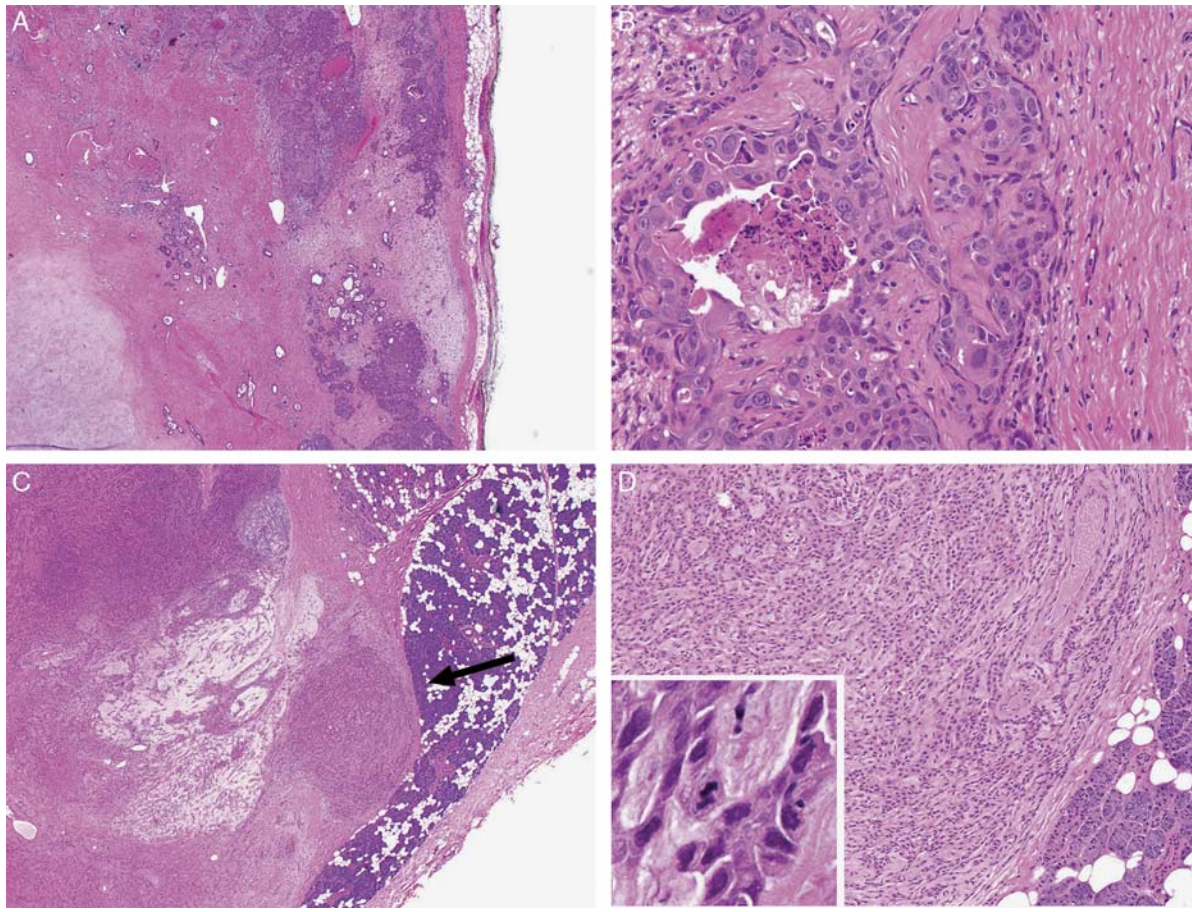

FIGURE 12. Architectural CAxPA variants. A, Intracapsular CAxPA showing a completely encapsulated tumor. The low power cue to potential worrisome features is the sclerosis within this PA (H&E 20 ×). B, Within these areas, a high-grade adenocarcinomatous component with comedonecrosis and apocrine features indicative of an intracapsular salivary duct carcinoma component is noted (H&E, 200 ×). C, Minimally invasive CAxPA with 1 minute focus (arrow) of extension no further than 1.5 mm beyond the tumor capsule (H&E, 20 ×). D, This focus is distinguished from a mere pseudopod in a cellular PA by the increased cellularity and sclerotic stroma. The carcinomatous component is a spindled myoepithelial carcinoma (H&E, 100 ×). Inset: The cells are monomorphic but mitotically active (H&E, 400 ×). CAxPA indicates carcinoma ex pleomorphic adenoma; H&E, hematoxylin and eosin.

Prognostic biomarkers in AciCC are scarce. One marker that may be of value is Ki-67 though this needs to be validated on larger series. In 1 initial study, all tumors with a Ki-67 proliferation index less than 5% behaved in a favorable manner.[75] One recent small series of AciCC show that p53, EGFR, and Her-2/Neu overexpression also predict an unfavorable outcome.[76]

Similar to ACC, AciCC can also undergo progression to a poorly differentiated or undifferentiated carcinoma (AciCC-HGT). The transformed component in AciCC typically consists of a solid to cribriform-patterned proliferation of pleomorphic tumor cells with comedonecrosis and often loss of acinar differentiation, namely absence of zymogen granules (Fig. 14). Rare cases of spindle cell/myoepithelial transformation have been noted.[77,78] The molecular pathogenesis is not well understood, but, unlike ACC-HGT, AciCC-HGT is not commonly associated with p53 alterations.[79,80] The largest single series to date also suggests that c-kit and Her-2/neu are also noncontributory toward HGT in AciCC, although cyclin-D1 may have a role.[80] Unlike conventional AciCC, AciCC-HGT is a uniformly aggressive disease with a median survival of only 4.3 years. Lymph node and distant metastases may be found in over 1/2 of cases.[80] Although difficult to be certain, it is possible that some of the "high grade" tumors in the larger series of AciCC may actually represent AciCC-HGT.[8,73]

CONCLUSIONS

Histologic grading in many salivary gland carcinoma types is prognostically valuable despite its shortcomings. In MECs grading is prognostically and therapeutically relevant. Standardized grading systems are more reproducible than generic grade assignment, but may be cumbersome and have room for refinement. The grading system is largely responsible for the confusing biologic behavior of intermediate grade MEC category is heavily dependent on the grading system used and is thus the most controversial with regards to management and prognosis. The t(11;19)(q21; p13) *MECT1-MAML2* translocation shows potential as an objective prognosticator. ACC is graded based on growth pattern with solid growth imparting a poorer prognosis, although therapeutically perhaps not as significant. Several chromosomal alterations hold promise for identification of new

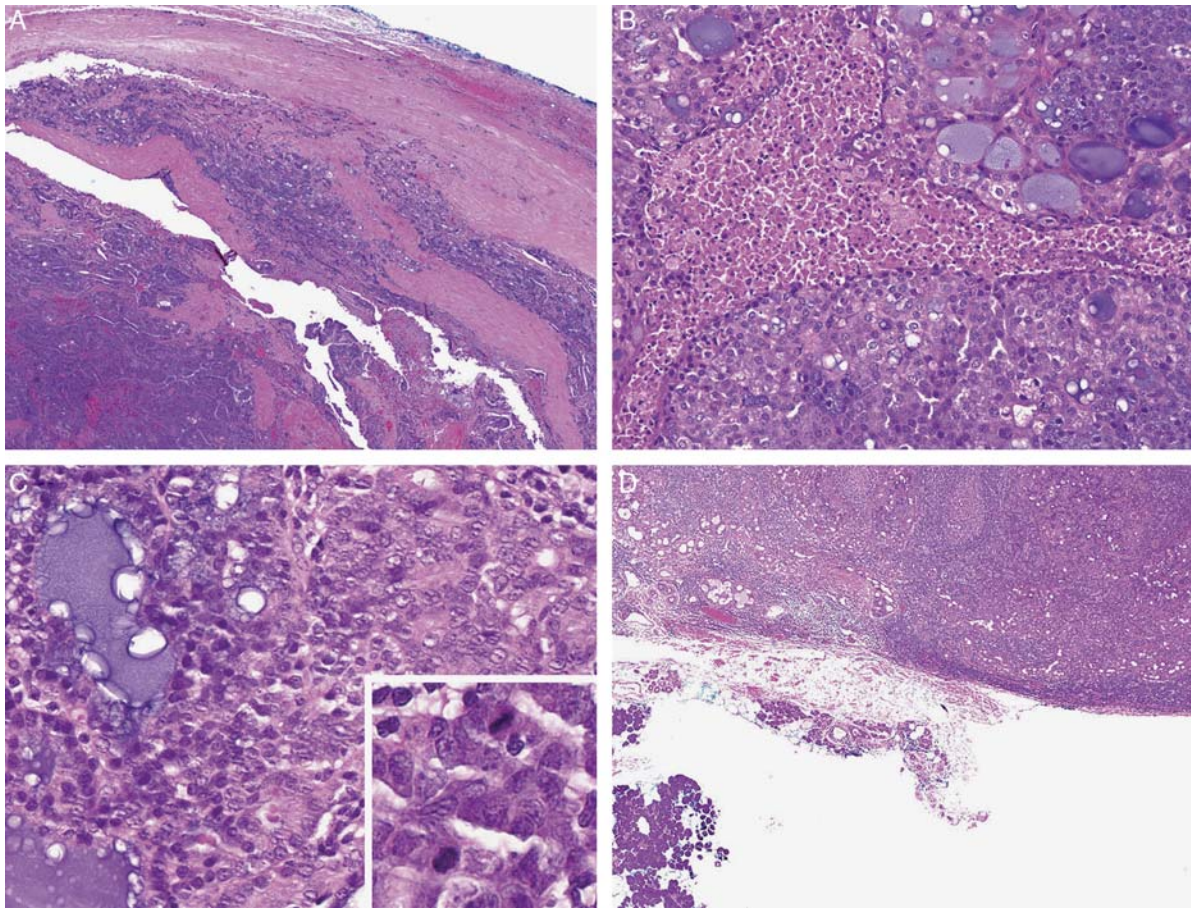

FIGURE 13. Histologic features in acinic cell carcinoma of potential prognostic value. A, Sclerotic stroma (H&E, 20 ×) which may be an adverse prognosticator. B, Necrosis (40 ×) also an aggressive parameter. C, Cytologic atypia is also intuitively considered aggressive. Often this is accompanied by loss of zymogen granules (right) (H&E, 400 ×). Inset (400 ×) showing increased mitotic activity. D, In contrast, a well-demarcated microcystic growth pattern with a prominent "lymph node-like" lymphoid architecture is thought to be a favorable finding (H&E, 400 ×). H&E indicates hematoxylin and eosin.

prognostic markers. Rarely, these tumors will undergo HGT in which case the tumor will have a much higher rate of lymph node metastases than a conventional ACC. CAxPA is a category rather than a specific entity. Behavior is determined by the carcinoma type, quantity, and extent. AciCC is typically a low-grade tumor, but evidence indicates

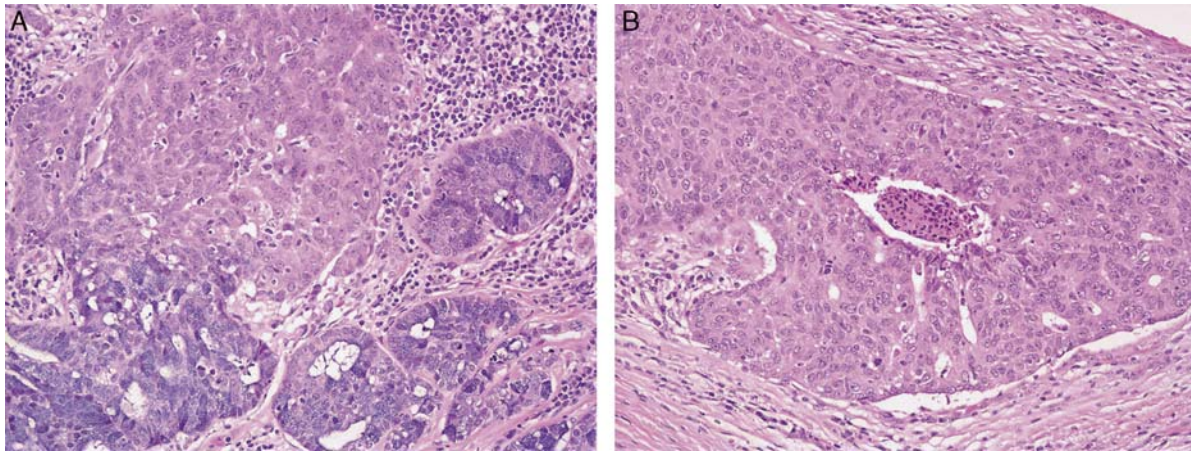

FIGURE 14. Acinic cell carcinoma with high-grade transformation. A, As the tumor transitions to a pleomorphic high-grade carcinoma, zymogen content is lost (H&E, 200 ×). B, In some areas, this component looks almost exactly like salivary duct carcinoma with pink cytoplasm, cribriform growth, and comedonecrosis (H&E, 200 ×). H&E indicates hematoxylin and eosin.

a dichotomous behavior that can be predicted by histologic parameters thus suggesting that grading of these tumors is feasible. AciCC can also undergo HGT which thus imparts a highly adverse prognosis.

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